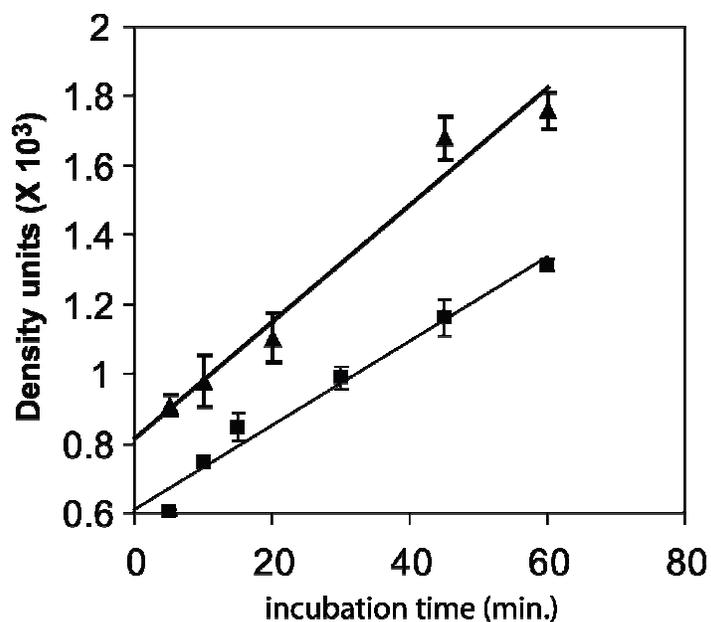


Supplementary Figure 1. Recognition of a noncognate T domain by AdmF. .

Shown are the 10+ charge states of EntB and AdmI. The (*S*)- β -Phe and octanoyl chains were loaded onto thiolation domains AdmI (peak 1) and EntB (peak 2), respectively. The AdmF reaction led to the formation of two products, octanoyl-(*S*)- β -Phe-S-AdmI (peak 3) and holo EntB (peak 4). Product formation was confirmed by observation of the octanoyl-(*S*)- β -Phe-S-pantetheine ejection product at m/z 534.2987 (theoretical: 534.2992, -0.9 ppm error).



Supplementary Figure 2. Apparent rate of isopeptide bond formation catalyzed by AdmF using the nucleophiles (*S*)-β-Phe (▲) and (*R*)-β-Phe (■). The reaction was carried out using 0.2 μM AdmF, 50 μM of [¹⁴C]-butyryl-*S*-AdmA and 50 μM GST-AdmI loaded with (*S*)-β-Phe or (*R*)-β-Phe. The reaction was followed by radio-SDS-PAGE. The resulting radiogram was analyzed using ImageQuant (Amersham). The transfer of the radioactive label to the AdmI thiolation domain was quantified using three separate analysis strategies (median intensity, average intensity and total pixel count). The average turnover rates of AdmF using substrates (*S*)-β-Phe and (*R*)-β-Phe were determined to be 16.8 and 12.1 density units/minute, respectively.